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Biochar and biochar with N-fertilizer affect soil N₂O emission in Haplic Luvisol

Ján HORÁK^{1*}, Elena KONDRLOVÁ¹, Dušan IGAZ¹, Vladimír ŠIMANSKÝ², Raphael FELBER³,
Martin LUKAC^{4,5}, Eugene BALASHOV⁶, Natalya BUCHKINA⁶, Elena Y. RIZHIYA⁶ & Michal
JANKOWSKI⁷

¹*Department of Biometeorology and Hydrology, Horticulture and Landscape Engineering
Faculty, Slovak University of Agriculture in Nitra, Hospodárska 7, 949 01 Nitra, Slovakia; e-
mail: jan.horak@uniag.sk*

²*Department of Soil Science, Faculty of Agrobiological and Food Resources, Slovak University
of Agriculture, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia*

³*Agroscope, Climate & Air Pollution, Reckenholzstrasse 191, 8046 Zürich, Switzerland*

⁴*School of Agriculture, Policy and Development, University of Reading, Reading RG66AR,
UK*

⁵*Department of Forest Management, Faculty of Forestry and Wood Sciences, Czech
University of Life Sciences, 16521 Prague, Czech Republic*

⁶*Agrophysical Research Institute, Grazhdansky pr. 14, St. Petersburg, 195220 Russia*

⁷*Department of Soil Science and Landscape Management, Faculty of Earth Sciences,
Nicolaus Copernicus University, Lwowska 1, 87-100 Toruń, Poland*

*Corresponding author.

Running Title: *Effect of biochar on soil properties, soil N₂O emissions and yield*

Abstract: The benefits of biochar application are well described in tropical soils, however there is a dearth of information on its effects in temperate soils used in arable agriculture. An

interesting and little explored interaction may occur in an intensive agriculture setting; biochar addition may modify the effect of commonplace N-fertilization. We conducted a field experiment to study the effects of biochar application at the rate of 0, 10 and 20 t ha⁻¹ (B0, B10 and B20) in combination with 0, 40 and 80 kg N ha⁻¹ of N-fertilizer (N0, N40, N80). We followed nitrous oxide (N₂O) emissions, analysed a series of soil physicochemical properties and measured barley yield in a Haplic Luvisol in Central Europe. Seasonal cumulative N₂O emissions from B10N0 and B20N0 treatments decreased by 27 and 25% respectively, when compared to B0N0. Cumulative N₂O emissions from N40 and N80 combined with B10 and B20 were also lower by 21, 19 and 25, 32%, respectively compared to controls B0N40 and B0N80. Average *pH* was significantly increased by biochar addition. Soil NO₃⁻ and NH₄⁺ concentrations were not affected by either biochar or N addition. There was a statistically significant increase of soil water content in B20N0 treatment compared to B0N0 control, possibly as a result of larger surface area and the presence of micropores having altered pore size distribution and water-holding capacity of the soil. application of biochar at the rate of 10 t ha⁻¹ had a positive effect on spring barley grain yield.

Key words: biochar; nitrogen fertilization; soil properties, N₂O emission, yield.

Introduction

Driven by climate change and population growth, human pressure on land even today results in continuous conversion of natural landscapes to agricultural use. Further, arable agriculture has been shown to deplete plant resources in soils dedicated to long-term agricultural use (Lal 2009). for these reasons, sustainable concepts combining increased food production and soil sustainability are urgently needed to lower the pressure on soils and to prevent negative environmental impacts of intensive agriculture. The use of mineral fertilizers has played a

significant role in increasing agricultural productivity over the last half century (Gruhn et al. 2000). However, the application of mineral (nitrogen) fertilizer has been shown to contribute to a number of environmental issues, including greenhouse gas (*GHG*) emissions, stream eutrophication, drinking water contamination (Delgado & Follett 2010; Sutton & van Grisen 2011) and contributing to more rapid organic matter mineralization (Liu et al. 2010). It is thus imperative to focus on improving soil condition, especially its soil organic matter (*SOM*) content, as *SOM* has been positively linked to soil fertility and health.

A number of studies have shown that biochar is a promising soil amendment material which has the potential to mitigate climate change through increasing soil organic carbon (*SOC*) content and by improving soil quality, thus contributing to higher yield from smaller area (Laird et al. 2010; Zhang et al. 2012). Application of organic materials such as biochar is reported to improve soil chemical (Liang et al. 2006), physical (Atkinson et al. 2010; Czachor & Lichner 2013) and biological properties (Lehmann et al. 2011), biochar has also been shown to increase crop yields, reduce *GHGs* and increase soil carbon sequestration (Lehmann et al. 2006). Biochar added to arable soils exerts some control over N dynamics (Clough et al. 2013) and has the potential to reduce *N₂O* emissions from soils (Hüppi et al. 2015). The meta-analysis of Cayuela et al. (2014) supports these findings, it shows a 54% reduction of *N₂O* emissions in laboratory and field studies. However, the evidence is not conclusive, some studies indicate opposite (Verhoeven & Six 2014), as well as no effect of biochar addition on soil *N₂O* flux (Suddick & Six 2013). Improved knowledge of the effects of biochar application to soils in agricultural context is thus still needed. Several studies on biochar addition focus on soils with deficient functionality and sub-standard yield potential (e.g. acid, saline, low *SOC* soils) where the changes after biochar application are expected to be robust. However, the likelihood of biochar application may be the greatest in fertile agricultural soils with the greatest economic and practical opportunity for biochar application. Highly productive soils may be able to offer an

economic return on biochar application, however careful attention still needs to be paid to economic risks linked with biochar price and its effects of soil fertility and crop yield.

Taking into account the above-mentioned concepts, the specific objective of this study was to quantify the effects of biochar and biochar combined with N-fertilizer application on N_2O emissions, soil physicochemical properties and crop yield in a Haplic Luvisol in a fully commercial setting. In particular, we set out to investigate if (**H1**) biochar addition reduces N_2O emission from arable soils, (**H2**) biochar addition is able to counter increased N_2O emission driven by N fertilisation and (**H3**) biochar addition has a positive effect on crop yield.

Materials and methods

Experimental site

The field experiment was established at the experimental site of Slovak University of Agriculture (Malanta) in the Nitra region of Slovakia (lat. 48°19'00''; lon. 18°09'00''). The study covered the period from March to November 2014, taking in the whole growing season of spring barley (*Hordeum vulgare* L.). The site is in the temperate zone, with a mean annual air temperature of 9.8 °C and mean annual rainfall of 539 mm. The mean air temperature and rainfall in 2014 was 10.3 °C and 640.8 mm, respectively. The field has been under conventional crop management for several years prior to this experiment. The soil is classified as Haplic Luvisol (WRB 2006), soil samples from soil depth of 0–10 cm at 10 random locations (experimental field trial) were taken prior to setting up the experiment to ascertain background conditions. On average, the soil contained 360.4 g kg⁻¹ of sand, 488.3 g kg⁻¹ of silt and 151.3 g kg⁻¹ of clay. SOC was 9.13 g kg⁻¹, while the average soil pH (KCl) was 5.71.

Experimental set-up

The experiment was established in March 2014, followed by biochar application (0, 10 and 20 t ha⁻¹) and N-fertilizer application (0, 40, 80 kg N ha⁻¹) as the main treatments (Table 1). The

replicated ($n = 3$) trial plots ($4 \text{ m} \times 6 \text{ m}$) were laid out in a randomized block design separated by a 0.5 m wide protection row. The entire experimental field was plowed prior to setting up the experiment, followed by randomly allocating treatments and finally by biochar and fertilizer application to the soil surface and their immediate incorporation into the 0-10 cm soil layer using a combinator. Spring barley was planted on 11th March 2014 at a commercial seed density of 200 kg ha^{-1} . All biochar used in this experiment was produced from paper fiber sludge and grain husks (1:1, Sonnenerde, Austria) by pyrolysis at 550°C for 30 minutes in a Pyreg reactor (Pyreg GmbH, Dörth, Germany). On average; it contained 57 g kg^{-1} of *Ca*, 3.9 g kg^{-1} of *Mg*, 15 g kg^{-1} of *K* and 0.77 g kg^{-1} of *Na* (DIN EN ISO 11 885). Total C content of biochar was 53.1 %, while total N content was 1.4 % (DIN 51732), the C:N ratio was 37.9, specific surface area (SSA) was $21.7 \text{ m}^2 \text{ g}^{-1}$ (DIN 66132/ISO 9277) and content of ash was 38.3 % (DIN 51719). On average, the biochar $pH(\text{CaCl}_2)$ was 8.8 (DIN ISO 10390). Calcium-ammonium nitrate was used as N fertilizer.

Soil sampling and analysis

Soil samples for soil pH , ammonium (NH_4^+) and nitrate (NO_3^-) measurements were taken monthly from each plot (March-October, 2014). Three randomly distributed soil cores (0 - 10 cm) per plot were taken at each soil sampling and pooled to produce an average representative sample. Samples were processed in the lab, soil pH was determined potentiometrically in 1 M KCl (1:2.5, soil:distilled water). Mineral N ($\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$) was extracted with 1% K_2SO_4 from field-moist soil. Amounts of soil $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ in isolates were determined using calorimetric method with spectrometer (WTW SPECTROFLEX 6100, Weilheim, Germany). Bulk density was measured right after application of treatments on 19th March and on 2nd May at a depth of 2-7 cm using a soil core (100 cm^3).

Nitrous oxide measurement

Soil air emission samples were taken between March and November 2014. A metal collar frame was inserted 10 cm deep into the soil in every plot treatment and left undisturbed until the next agronomic intervention, when it was lifted and replaced in the original location. Gas sampling took place at weekly intervals, the chambers (30 cm in diameter and 25 cm in height) were water-sealed onto bottom collars at every sampling event and gas samples were collected through tube fittings (20 ml, sealed with septum) at 0, 30 and 60 minutes after chamber deployment using an air-tight syringe (Hamilton) and transferred to pre-evacuated 12 ml glass vials (Labco Exetainer). Gas samples were analyzed for N_2O using a gas chromatograph (GC-2010 Plus Shimadzu), equipped with electron capture detector (ECD). Soil water content (SWC) at 0-10 cm depth (gravimetric method) and soil temperature at 5 cm depth (Volcraft DET3R thermometer) were also measured at each gas sampling event.

Plant sampling and analysis

Sampling of plant biomass was carried out in a quadrat (0.5 x 0.5 m), randomly positioned within each plot at the end of the growing season on July 14th, 2014. Total plant biomass was transported to the lab, where the plants were counted and roots separated from above-ground biomass. Ears were separated from stems and counted. Grain was threshed in a mechanical thresher and counted by a digital seed counter. The grain and the rest of above ground biomass were dried separately in the oven at 60 °C at least for 5 days until dry weight and then weighted. Final grain yield was calculated as a multiplication of total number of ears per m², number of grains per ear and average grain weight at 85% of dry biomass (HGCA 2005).

Statistical analysis

One-way analysis of variance (ANOVA) and the least significant difference (LSD) method was used to compare treatment means for the two levels of biochar and three levels of nitrogen application at $p < 0.05$. The analyses were performed using the Statgraphics Centurion XV.I programme (Statpoint Technologies, Inc., USA).

Results and discussion

Soil physicochemical properties

Soil physical and chemical properties averaged over the whole of the growing season are presented in Table 2. Generally, all biochar addition treatments (10 and 20 t ha⁻¹) increased soil *pH* at all sampling dates (data not shown), with the average *pH* over the duration of the experiment increasing significantly in biochar addition treatments when compared to those with no biochar. The *pH* values correlated significantly with the biochar application rate in the following order B0N0 < B10N0 < B20N0. The same trend was observed when no nitrogen was applied, but also in the treatments fertilized with 40 and 80 kg N ha⁻¹. Other studies confirm this finding, an increase of *pH* was shown when biochar with *pH* higher than that of the soil was applied (Yuan et al. 2011b). Similarly, a clear increase of soil *pH* with increasing biochar application rate was shown by Yuan et al. (2011a), but also by other studies (Atkinson et al. 2010; Zhang et al. 2012; Jones et al. 2012). The increase in soil *pH* caused by organic material amendments was mainly attributed to organic anions present in added materials, as indicated by the concentration of excess cations over inorganic anions, also termed ash alkalinity (Yan et al. 1996). One of the mechanisms put forward is decarboxylation of organic anions present in biochar, a process known to consume protons within the soil.

There was no difference in mean seasonal soil NO_3^- and NH_4^+ concentration between any of the treatments. Generally, mean soil NH_4^+ was higher in fertilized treatments when compared to those with no fertilization. Soil NH_4^+ content was influenced by fertilizer

application but not by biochar which confirms the findings of Appel & Klein (2015) who found that biochar had no relevant effect on soil NH_4^+ content. Our results show slightly higher NH_4^+ concentration in both biochar addition treatments as compared to control when no nitrogen was applied. The same trend was found in B10N80 compared to its fertilization level control (B0N80). However, NO_3^- availability in a combined biochar and nitrogen treatment was lower than in the N addition only. Here, our data agree with studies that report a decrease of NO_3^- concentration after biochar addition to soil (Ippolito et al. 2012; van Zwieten et al. 2010). Smaller NO_3^- availability has been attributed to microbial immobilization after biochar addition (Ippolito et al. 2012; Singh et al. 2010), which could also be our case (Table 2).

The average SWC was improved by biochar amendment (10 and 20 t ha⁻¹) in all nitrogen fertilizer treatments (0, 40, and 80 kg N ha⁻¹). However, statistically significant improvement was found only in B20N0 compared to B0N0. Our findings on SWC are in line with recent studies (Barrow 2012; Agegnehu et al. 2015; Leelamanie 2014; Liyanage & Leelamanie 2016) which report that organic amendments enhance soil water retention capacity. Biochar, with its large surface area and micropore abundance, does alter mean soil particle surface area, pore size distribution and thus WHC of the soil (Chintala et al., 2014a). Incorporation of biochar may enhance specific surface area up to 4.8 times compared to unadulterated soils (Liang et al., 2006) and may also increase the presence of capillary pores.

Soil bulk density in the middle of the growing season was lower in the biochar amended plots and at all fertilization levels, as compared to the control plots. This is consistent with a number of studies which have also found biochar amendment to reduce soil bulk density (Schnell et al. 2012; Case et al. 2012; Zhang et al. 2010). However, overall bulk density was not affected by the treatments, the only significant differences having been observed at the beginning of the experiment between B0N40 and B20N40 and between B10N40 and B20N40.

This indicates that a higher dose of biochar in treatments with 40 kg N ha⁻¹ significantly improved bulk density. However, we assume that this was not the effect of N fertilization, but just the impact of higher dose of biochar at this treatment

Nitrous oxide emissions

N₂O emissions in all treatments were the highest during the initial 4 weeks after trial establishment, but episodically during several peak events in the summer, with steady background emissions occurring during the rest of the season (**H1**, Fig. 1a). The bulk of *N₂O* flux has occurred shortly after crop harvest and disking of all plots. All treatments showed similar temporal *N₂O* emissions dynamics, but the heights of the peaks did differ. Almost all emissions peaks observed in the biochar treatments were lower than those with no biochar. The results of this study show that mean seasonal *N₂O* emission in all three N-fertilization levels (0, 40 and 80 kg N ha⁻¹) were higher when compared to treatments which included biochar application (10 and 20 t ha⁻¹) (**H2**, Table 2, Fig. 1b), a result in accordance with that of Liu et al. (2012). However, differences among treatments were not always statistically significant due to the high variability among the replicates. Both biochar treatments (B10N0, B20N0) significantly reduced *N₂O* emissions, compared to the control treatment (B0N0). The plots fertilized with 80 kg N ha⁻¹ show that only the higher application rate of biochar is sufficient to significantly reduce *N₂O* emission. Spatial variability within and among the plots could be a factor contributing to the non-conclusiveness of results, as reported in the study of Fangueiro et al. (2008).

Lower emissions peaks from plots with biochar amendments resulted in an increasing difference in cumulative fluxes between biochar plots and control plots over the duration of the trial (Table 2, March-November, 2014). By the end of the experiment, compared to B0N0, cumulative *N₂O* emission from plots amended with 10 and 20 t ha⁻¹ of biochar (B10N0, B20N0)

were reduced by 27 and 25%, respectively. The cumulative fluxes from fertilized plots at 40 and 80 kg N ha⁻¹, combined with 10 and 20 t ha⁻¹ of biochar were also lower by 21, 19 and 25, 32%, in comparison to their respective controls B0N40 and B0N80. A study similar to ours has reported that *N₂O* emissions were between 26% and 79% lower in biochar treated plots than in control plots (Castaldi et al. 2011). On the other hand, there are observations of non-significant effects of biochar application on *N₂O* emission (Karhu et al. 2011; Anderson et al. 2011). Further, Shen et al. (2014) found that biochar amendment of a rice field increased *N₂O* emissions compared to an NPK only treatment, although the last observation relates to anoxic soil conditions of a rice paddy.

The mechanisms explaining the observed reduction of *N₂O* emissions following biochar application are still uncertain. In aerobic soils, *N₂O* is primarily a byproduct of nitrification (NH₄⁺ to NO₃⁻) and to a lesser extent of anaerobic denitrification (NO₃⁻ to N₂). Nitrogen availability strongly affects both processes and in arable soils is directly related to N fertilizer addition or the organic N content of the soil. Biochar-induced changes in N availability and enhanced plant uptake may reduce *N₂O* emission for soils (Steiner et al. 2007). In this study, monthly soil sampling showed that the seasonal soil NO₃⁻ and NH₄⁺ was not significantly different between any of the treatments (data not shown). However, we observed a short-lived decrease of NO₃⁻ content after biochar addition to soil, as well as a corresponding decrease of *N₂O* flux, which suggests that N availability reduced by biochar is one of the mechanisms responsible for decreasing *N₂O* emissions.

We have observed higher average *pH* in biochar amended soils, a result similar to findings of other studies (Atkinson et al. 2010; Singh et al. 2010). Since soil *pH* exerts control over the N₂O : N₂ ratio during denitrification (Simek & Cooper 2002), a higher *pH* seen in biochar treatments might also contribute to the reduction of *N₂O* emissions.

Crop yields

The application of 10 t ha⁻¹ of biochar increased final grain yield at all fertilization levels, however significant difference was found only between B10N0 and B0N0 (Table 3, **H3**). Combining 40 kg N ha⁻¹ fertilizer with biochar (both application rates) significantly increased the number of plants per m² by 31% on average. Biochar application combined with 80 kg N ha⁻¹ decreased the amount of plants per m², but led to a larger aboveground biomass and grain yield when compared to B0N80 control. This effect could be an indicator of positive impact of biochar on yield development during grain filling, as suggested by Agegnehu et al. (2016). Biochar applied together with 40 kg N ha⁻¹ fertilizer increased average single grain weight by 7 and 19% in B10N40 and B20N40 treatments, respectively. An increase of 3% was observed also for non-fertilized treatment (B20N0). However, the 80 kg N ha⁻¹ fertilizer showed no effect on single grain weight. These results from the first year of experiment are consistent with findings of other studies looking at the effect of biochar application on spring barley (Nelissen et al. 2015; Karer et al. 2013).

Conclusions

A significant responses of soil N_2O emissions, soil pH , soil water content, bulk density and yield parameters to biochar and biochar combined with nitrogen fertilizer application are reported in this study. Biochar amendment of Haplic Luvisol under arable regime shows its potential to reduce N_2O emissions, increase soil pH , but showed no effect on soil NO_3^- and NH_4^+ . The highest increase of pH and soil water content was found when 20 t ha⁻¹ of biochar was applied. Barley grain yield significantly increased only after application of 10 t ha⁻¹ of biochar. From the standpoint of, Biochar and biochar combined with nitrogen fertilization appears to be a promising practice to improve sustainability of intensive agriculture by lowering N_2O emissions and improving soil water retention. In addition, a certain level of mineral N

immobilization and increased soil *pH* can be achieved. However, more research is needed on different soil types at different agro-ecosystems beyond one year before this practice is fully recommended to farmers.

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Table 1. Treatments including individual amounts of applied N-fertilizers (1st column) and biochar (2nd, 3rd, 4th column)

Amount of N-fertilizer application level (N) kg ha ⁻¹	Amount of biochar applied (B) t ha ⁻¹		
	0	10	20
0	B0N0	B10N0	B20N0
40	B0N40	B10N40	B20N40
80	B0N80	B10N80	B20N80

Table 2. Effect of biochar treatments on soil physicochemical properties and N₂O emissions averaged over the whole of the growing season

Treatments	pH (KCl)	NH ₄ ⁺ mg kg ⁻¹	NO ₃ ⁻ mg kg ⁻¹	BD Trial- start (g cm ⁻³)	BD Trial- mid (g cm ⁻³)	SWC (%)	N ₂ O (g N ₂ O-N ha ⁻¹ day ⁻¹)	Cumulative N ₂ O (g N ₂ O-N ha ⁻¹ 8 months ⁻¹)
Not fertilized								
B0N0	5.25 a	6.39 a	3.88 a	1.39 a	1.33 a	16.2 a	7.26 b	1725b
B10N0	5.64 b	6.40 a	3.56 a	1.35 a	1.30 a	16.6 ab	5.02 a	1267a
B20N0	5.88 c	6.91 a	3.54 a	1.28 a	1.27 a	17.9 b	5.16 a	1288a
40 kg N ha ⁻¹								
B0N40	5.16 a	8.56 a	4.19 a	1.43 b	1.28 a	16.1 a	6.97 a	1662a
B10N40	5.86 b	7.82 a	4.01 a	1.37 b	1.24 a	16.9 a	5.27 a	1317a
B20N40	5.87 b	7.48 a	3.51a	1.22 a	1.09 a	17.8 a	5.37 a	1345a
80 kg N ha ⁻¹								
B0N80	5.08 a	9.09 a	5.31 a	1.34 a	1.28 a	16.2 a	9.12 b	2311b
B10N80	5.67 b	9.41 a	3.63 a	1.42 a	1.14 a	16.9 a	6.94 ab	1744ab
B20N80	5.97 c	8.19 a	3.80 a	1.24 a	1.19 ab	17.7 a	6.27 a	1562a

Different letters between row indicate that treatment means over the sampling dates are significantly different at $P < 0.05$ according to LSD multiple-range test. Note: BD: soil bulk density.

Table 3 Effect of biochar and fertilizer on crop yield parameters (means \pm standard error; n=3). Different letters indicate significant difference at $P < 0.05$ according to LSD multiple-range test

Treatments	Number of plants m ²	Above-ground dry biomass t ha ⁻¹	Average single grain weight at 85% DM mg	Final grain yield at 85% DM t ha ⁻¹
Not fertilized				
B0 N0	223 \pm 26.8 a	8.0 \pm 1.2 a	43.3 \pm 0.7 a	3.6 \pm 0.8 a
B10N0	221 \pm 18.5 a	10.8 \pm 2.1a	43.0 \pm 0.1 a	5.1 \pm 0.9 b
B20N0	209 \pm 41.5 a	7.1 \pm 0.8 a	44.6 \pm 0.7 a	3.2 \pm 0.5 a
40 kg N ha ⁻¹				
B0N40	172 \pm 10.6 a	8.4 \pm 0.5 a	42.0 \pm 1.1 a	3.7 \pm 0.5 a
B10N40	225 \pm 11.4 b	8.2 \pm 0.1 a	45.1 \pm 1.1 a	3.9 \pm 0.2 a
B20N40	227 \pm 14.8 b	7.9 \pm 0.9 a	49.9 \pm 5.2 a	3.6 \pm 0.5 a
80 kg N ha ⁻¹				
B0N80	200 \pm 19.7 a	10.8 \pm 0.7 a	43.8 \pm 1.2 a	5.0 \pm 0.3 a
B10N80	189 \pm 10.9 a	11.4 \pm 2.1 a	42.2 \pm 1.4 a	5.4 \pm 0.9 a
B20N80	183 \pm 15.4 a	10.3 \pm 0.7 a	43.4 \pm 0.8 a	4.9 \pm 0.4 a

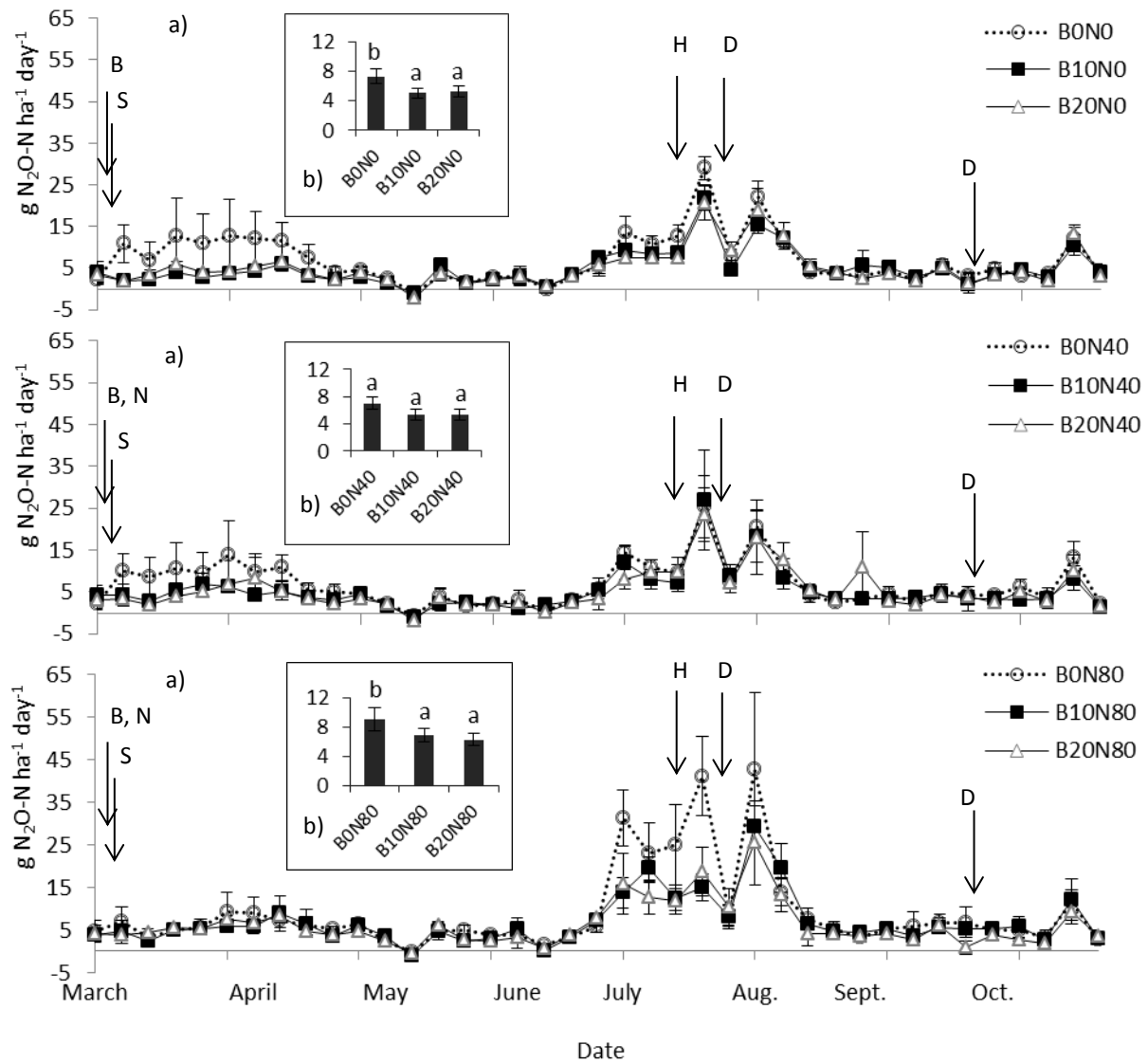


Fig. 1. a) Temporal changes of N_2O emissions from control and biochar amended soil plots during the field trial period. Error bars represent $\pm\text{SE}$. B - biochar application; N - nitrogen fertilizer application; S - sowing of spring barley; H - harvesting spring barley; D – disking. b) average N_2O emissions at different treatments over the field trial period. Error bars represent the standard errors among the average data of the sampling dates.